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Genetic Variation of a *DRD2* Co-Expression Network is Associated with Changes in Prefrontal Function After D2 Receptors Stimulation

Pierluigi Selvaggi[1,2,‡], Giulio Pergola[1,‡], Barbara Gelao[1], Pasquale Di Carlo[1], Maria Antonietta Nettis[1,3], Graziella Amico[1], Leonardo Fazio[1], Antonio Rampino[1,5], Fabio Sambataro[4], Giuseppe Blasi[1,5], Alessandro Bertolino[1,5]

[1]Group of Psychiatric Neuroscience, Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari Aldo Moro, Bari, Italy

[2]Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

[3]Department of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

[4]Department of Experimental and Clinical Medical Science, University of Udine, Udine, Italy

[5]Azienda Ospedaliero-Universitaria Consorziale Policlinico, Bari, Italy

[‡]Designates equal contribution as first authors

Corresponding author: Alessandro Bertolino
Department of Basic Medical Science, Neuroscience and Sense Organs
University of Bari Aldo Moro
Piazza Giulio Cesare, 11, I-70124, Bari, Italy
alessandro.bertolino@uniba.it
tel +39 080 5478572
fax +39 080 5593204

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Abstract

Dopamine D_2 receptors contribute to the inverted U-shaped relationship between dopamine signalling and prefrontal function. Genetic networks from post-mortem human brain revealed 84 partner genes co-expressed with *DRD2*. Moreover, eight functional single nucleotide polymorphisms combined into a polygenic co-expression index (PCI) predicted co-expression of this *DRD2* network and were associated with PFC function in humans. Here, we investigated the non-linear association of the PCI with behavioural and WM-related brain response to pharmacological D_2Rs stimulation.

Fifty healthy volunteers took part in a double-blind, placebo-controlled, fMRI study with bromocriptine and performed the N-Back task. The PCI×drug interaction was significant on both WM behavioural scores (p=.046) and related PFC activity (all corrected p<.05) using a polynomial PCI model. Non-linear responses under placebo were reversed by bromocriptine administration. fMRI results on placebo were replicated in an independent sample of 50 participants who did not receive drug administration (p=.034).

These results match earlier evidence in non-human primates and confirm the physiological relevance of this *DRD2* co-expression network. Results show that in healthy subjects different alleles evaluated as an ensemble are associated with non-linear PFC responses. Therefore, brain response to a dopaminergic drug may depend on a complex system of allelic patterns associated with *DRD2* co-expression.

Introduction

Converging evidence from animal and human studies indicates that dopamine (DA) plays a key role in Working Memory (WM) (Williams and Goldman-Rakic 1995; Murphy et al. 1996; Mattay et al. 2000; Mehta et al. 2000; Chudasama and Robbins 2004; Zhang et al. 2007; Sambataro et al. 2009; Cassidy et al. 2016). DA is thought to exert its effects on WM by regulating neuronal firing rates in recurrent circuits of the prefrontal cortex (PFC) (Goldman-Rakic 1995; Seamans and Yang 2004). Animal models show that the dose-dependent relationship between DA signalling and neuronal firing rates in the PFC follows an inverted-U shaped response (Vijayraghavan et al., 2007). In the PFC, DA binds D_1 and D_2 receptors (D_2Rs) of pyramidal neurons and GABA interneurons (Durstewitz et al. 2000; Seamans and Yang 2004; Avery and Krichmar 2015). In particular, based on evidence from animal and human studies (Seamans and Yang 2004; Kahnt et al. 2015), D_2Rs are thought to promote the maintenance of multiple and concurrent representations, although excessive D_2R-mediated signalling has been proposed to decrease signal to noise ratio in the PFC. In line with this proposal, behavioural studies in humans have shown that D_2R agonists like bromocriptine and pergolide and antagonists like sulpiride and haloperidol modulate behavioural WM performance (Luciana et al. 1992; Luciana and Collins 1997; Mehta et al. 1999; 2001; Kimberg and D'Esposito 2003).

Also inter-individual differences in WM performance are thought to depend on DA signalling in the PFC (Kimberg et al. 1997; Slifstein et al. 2015). Across subjects, intermediate levels of DA signalling are associated with optimal WM performance, while excessively low and high DA signalling are associated with suboptimal performance (Williams and Castner 2006; Cools and D'Esposito 2011). Inter-individual variation in WM performance is related with individual WM capacity, i.e., the limited amount of memory representations that can be

maintained and updated (Wilhelm et al. 2013). WM capacity and WM-related prefrontal activity have a heritability estimated up to 40 percent (Blokland et al. 2011; Fletcher et al. 2014; Hansell et al. 2015); particularly for the N-back task see (Blokland et al. 2008; Vogler et al. 2014) and are associated with genetic variation in DA-related genes (reviewed by (Karlsgodt et al. 2011). For instance, it has been previously shown that functional genetic variants such as *DRD2* rs1076560 and *COMT* Val158Met, which may modulate DA signalling, are also associated with WM performance and related brain activity (Zhang et al. 2007; Bertolino et al. 2008; Sambataro et al. 2009; Cohen et al. 2016; Luykx et al. 2017). Previous evidence also shows a DA-related genetic component of WM variation in response to stimulation with dopaminergic drugs (Mattay et al. 2003; Gelao et al. 2014).

However, previous studies have revealed that the genetic component of WM is complex (Blokland et al. 2016). Most functional genetic variants in the genome are in non-coding regions and are associated with epigenetic mechanisms of gene regulation. The regulation of gene expression is operated by transcription factors and non-coding RNAs affecting multiple genes, and thus multiple genes converge into co-expression pathways (Gaiteri et al. 2014). These pathways are reflected in co-expression patterns (Eisen et al. 1998). In this regard, we recently employed co-expression networks to analyse the complex genetic component of DA-related system-level phenotypes (Pergola et al. 2017). We examined the co-expression partners of *DRD2* and identified a cluster of 84 genes co-expressed with *DRD2*. Then, we identified functional single nucleotide polymorphisms (SNPs) predicting expression of the whole *DRD2* network and we combined them into a polygenic co-expression index (PCI) – thus indexing *DRD2* gene set co-expression. Critically, the PCI/co-expression relationship was replicated in an independent post-mortem dataset. We also provided biological validation of the PCI by investigating its association with PFC function during WM in healthy individuals and with clinical response to D_2R blockade in patients with schizophrenia. Both

findings were replicated in independent samples. Notably, previous studies investigated the effects on brain response to pharmacological stimulation of *single* functional SNPs within candidate genes (Jacobsen et al. 2006; Kirsch et al. 2006; London et al. 2009; Park et al. 2012; Gelao et al. 2014; Kasparbauer et al. 2014). However, the cumulative effect of multiple alleles has been reported to increase the effect size in association studies, including imaging genetics studies (Mattingsdal et al. 2013; Walton et al. 2013; Dima and Breen 2015; Pergola et al. 2016), even though the molecular mechanisms of SNPs are still unknown. While the evidence from our previous study supports the combination of multiple SNPs to investigate the neurobiology of WM, it is unclear whether the ensemble of these functional SNPs can be used to predict how the same individual responds to pharmacological challenges in a double-blind trial.

Here, we explored whether the PCI interacts with the effect of D_2R targeting drugs on WM capacity and related brain activity. Since WM capacity in humans is limited, inter-individual differences emerge most clearly when considering individual performance at multiple, challenging WM loads (Callicott, Mattay, Bertolino, Finn, Coppola, Frank, Goldberg, and Weinberger 1999a; Van Snellenberg et al. 2015). Thus, we investigated whether individual performance at multiple loads reflecting inter-individual heterogeneity in WM capacity is related with genetic variation in genes involved in *DRD2* co-expression.

In particular, we tested in healthy humans, thus independently of any disease-specific pathophysiological confound, whether allelic patterns of co-expression partner genes of *DRD2* are associated with behavioral and brain responses to D_2R stimulation during WM following an inverted U-shaped model (Cools and D'Esposito 2011). We hypothesized that individual response to D_2R targeting drugs in terms of WM capacity and modulation of PFC activity depends upon a complex individual genetic background co-expressed with *DRD2*. We performed a cross-over, double-blind, placebo controlled, randomized, genetic study with

bromocriptine (BRO) administration, a D_2R agonist with high affinity (Sautel et al. 1995). We indexed inter-individual variability in WM as the difference between the 3-back and 2-back in behavioural accuracy (percent of correct response) as well as its underlying prefrontal activity (Δ_{WM}). This approach is intended to obtain a metric with greater variability across individuals, because n-back accuracy is prone to ceiling effects (Callicott, Mattay, Bertolino, Finn, Coppola, Frank, Goldberg, and Weinberger 1999b; Van Snellenberg et al. 2015), which may bias the association with a continuous genetic index. Since differential accuracy is associated with the increase of cognitive load, less negative values are interpreted as representing greater WM capacity, i.e., more consistent accuracy in the face of load increase.

It is not possible to estimate DA concentration in the PFC based on genetic markers, but given the inverted U-shaped relationship between WM and DA signalling, we hypothesized that the effect of BRO on Δ_{WM} would depend on a quadratic term of the PCI reflecting *DRD2* co-expression. Currently available data support the inverted U-shaped model with DA concentration and D_1 stimulation (Seamans and Young, 2004), as well as with genetically predicted availability of NMDA subunits (Pergola et al., 2016), but this is the first study to test this model with regard to D_2Rs. Since DA acts on multiple neural pathways, we provided further systems-level validation of the findings outside the PFC. To this aim, we tested the association of the PCI with blood prolactin levels, which are modulated by D_2Rs and affected by BRO administration (Berry and Gudelsky 1991). Finally, we replicated the results obtained in the placebo condition in an independent dataset we reported previously (Pergola et al. 2017).

Materials and Methods

Participants

Seventy-one healthy volunteers (34 males, age mean±SD 26.4±5.1 years; 36 overlapping with the sample tested by (Gelao et al. 2014) were enrolled in the bromocriptine study. Before entering the study, all participants underwent a screening visit in which inclusion and exclusion criteria were evaluated. A medical assessment was performed by a trained physician, including medical history, physical exam and blood testing. Inclusion criteria were the absence of any psychiatric disorder, as evaluated with the Structured Clinical Interview for DSM-IV (First et al. 1997), Intelligence Quotient (IQ) > 80, as evaluated with the Wechsler Adult Intelligence Scale-Revised (Wechsler 1981), and age between 18 and 65 years. Since differential accuracy is not recommended as a WM performance index for participants with low accuracy (Cassidy et al., 2016), we only included individuals with above-chance accuracy at all loads considered (see Supplementary Materials, Section 3). Exclusion criteria were any neurological or medical condition considered as clinically significant or possibly interfering with the study by the physician during the screening visit, history of head trauma with loss of consciousness or drug abuse, hyperprolactinemia and any continuous pharmacological treatment in the past month. Substance abuse and substance dependence conditions (including alcohol) was excluded via a semi-structured interview based on DSM-IV-TR (SCID). Additional exclusion criteria were pregnancy or breastfeeding and use of oral contraceptives for female participants. On the same occasion, demographic and neuropsychological data were collected. In particular, the Hollingshead Scale (Hollingshead and Redlich 1958) was used to measure the socioeconomic status and the Edinburgh Inventory (Oldfield 1971) to evaluate handedness.

All subjects were unrelated Caucasians from the region of Apulia, Italy. After detailed description of the protocol, participants provided written informed consent according to the Declaration of Helsinki. The protocol was approved by the local ethics committee.

The entire sample was used for the analysis on prolactin peripheral levels. fMRI and behavioural analyses were performed on a subsample of 50 participants (27 males, mean age ± SD, 27.0 ± 4.1 years), who had no missing structural and functional MRI data and whose scans were considered adequate after a technical quality check (Supplemental Material and Methods, Section 3). Excluded participants did not differ with the analysis sample in terms of sociodemographic characteristic, IQ and PCI (all $p > .1$). Table 1 shows the sociodemographic characteristics of the sample.

In order to replicate part of the results obtained in the present study, we re-analysed data from our previous study (see Pergola et al. 2017 for details). In particular, we selected all participants from the fMRI study who performed the 3-back task (50 participants; see table 1).

Table 1 about here

Experimental procedure in the bromocriptine study

Participants underwent a double blind, randomized, placebo controlled, crossover trial with oral administration of BRO 1.25 mg as previously described (Gelao et al. 2014). Briefly, participants were scanned twice (two weeks apart), once after administration of BRO and once after lactose placebo (PLA). The order of administration, i.e., PLA first or BRO first,

was counterbalanced across participants. BRO and PLA were administered orally in identical capsules 150 minutes before the fMRI scan according to previous estimations of the time to reach peak of plasma concentrations (90-180 minutes) and of elimination half-life (Kvernmo et al. 2006). Domperidone (a peripheral selective D_2 antagonist which does not cross the blood-brain barrier, (Shindler et al. 1984) 10 mg was administered orally 30 minutes before both BRO and PLA to prevent possible side effects induced by BRO intake. The dosage of 1.25 mg was chosen as previous studies consistently reported that it was able to modulate behaviour and BOLD signal during WM and was not associated with significant adverse events (Luciana et al. 1992; Mehta et al. 2001). Nobody spontaneously reported any adverse event on study days.

Weighted Genes Co-Expression Network Analysis and Polygenic Co-Expression Index.

A polygenic co-expression index (PCI) was calculated as described in detail elsewhere (Pergola et al. 2017). Briefly, the Braincloud *post mortem* dataset (http://braincloud.jdmi.edu/; (Colantuoni et al. 2011) was used to perform a Weighted Genes Co-expression Network Analysis (Bin Zhang and Horvath 2005) in order to identify a *DRD2* co-expression gene set. This gene set included 85 genes (Table S1). A set of eight SNPs (Table 2) was significantly enriched for gene regulation function in the dorsolateral prefrontal cortex (DLPFC). The SNPs were associated with the co-expression of the entire gene set. The PCI was computed by assigning a weight to each genotype of each SNP based on the co-expression profile of the gene set, such that greater PCI corresponded to greater predicted gene set co-expression for that individual (Pergola et al. 2016; 2017). Ethnicity, population stratification and age effects on the interaction between the PCI and *post mortem* gene expression were assessed elsewhere (Pergola et al. 2017). Our previous work has shown that

population stratification effects are negligible in this sample, likely because the catchment area was limited and only native Caucasians born in Apulia were included in the study. Furthermore, the association of the PCI with *DRD2* co-expression was replicated in an independent *post mortem* dataset (BrainEAC, (Trabzuni et al. 2011). All participants were genotyped for the SNPs identified by (Pergola et al. 2017) to compute the PCI for each individual (for further details please see Supplemental Material and Methods and (Pergola et al. 2017).

Table 2 about here

Working Memory task

During fMRI, participants performed two runs of a block design WM task: the N-back task (Blasi et al. 2015). Stimuli consisted of numbers (1-4) shown in a random sequence and displayed at the points of a diamond-shaped box. In the WM condition participants were required, at each trial, to press the button corresponding to the stimulus seen two (2-Back), or three stimuli (3-Back) previously presented, while keeping on encoding incoming stimuli. The non-memory control condition required to identify the stimuli currently presented (0-Back). Both 2-Back and 3-Back were carried out by participants in two separate runs. In particular, each run consisted of 8 blocks of 30 seconds each. For the 2-Back run, four blocks of 0-Back (our control condition) were interleaved with the same number of 2-Back blocks (our experimental condition). This structure was identical for the 3-Back run. The same procedure has been followed for the participants in the replication sample (Pergola et al. 2017). For each session, before entering the scanner, all participants performed a practice

session of both task runs to achieve stable performance.

Behavioural data analysis

We computed differential accuracy (from now also called behavioural Δ_{WM} index) as the difference between 3- and 2-Back accuracy for both the bromocriptine and the replication samples and assessed whether it differed from zero using separate one-sample t-tests for the BRO and PLA conditions. We computed a repeated measures ANCOVA within SPSS (IBM SPSS Statistics, Version 22.0) to test the interaction between the PCI and BRO administration on behavioural data. Behavioural Δ_{WM} index was the dependent variable, *drug* (BRO or PLA) was the repeated measures factor, gender was a between-subjects factor, linear, quadratic, and cubic terms (to ensure that the quadratic fit was indeed the best fit) of the PCI were the continuous predictors. We used the quadratic term of the PCI to test for the inverted U-shaped associations of behavioural Δ_{WM} index with predicted transcription levels of the *DRD2* co-expression network, as indexed by the PCI. Since we used a polynomial model, we also introduced first- (linear) and zero-degree (constant) terms to marginalize the quadratic term for lower degree effects. To test the robustness of our analysis reducing the effect of extreme observations we used the bias corrected accelerated bootstrap technique with 10,000 resamples, bias-corrected accelerated algorithm (Efron and Tibshirani 1986). A comparison of fits analysis (quadratic vs linear and cubic vs quadratic) was performed on the differences between BRO and PLA in behavioural Δ_{WM} index marginalized by gender (Supplementary Materials, Section 4). Additionally, the Supplementary Material (Section 2) reports the results of further analyses addressing percent accuracy and reaction times separately by load.

In the replication sample, we computed a general linear model with the behavioural Δ_{WM}

index as the dependent variable, gender as a between-subjects factor, and the three polynomial terms of the PCI as continuous predictors. Following the results of the bromocriptine study, we estimated the t-parameter of the negative relationship between the quadratic PCI and behavioural the Δ_{WM} index and computed one-tailed p-values, as we had a priori evidence on the negative relationship.

fMRI data acquisition and analysis

fMRI data were acquired for both the bromocriptine and the replication sample with a 3T MRI scanner (SIGNA, GE Healthcare) with a gradient-echo planar imaging (EPI) sequence and the following parameters: repetition time= 2,000 ms; echo time= 28 ms; 20 interleaved axial slices; thickness= 4 mm; gap= 1 mm; voxel size, 3.75 × 3.75 × 5 mm; flip angle= 90°; field of view= 24 cm; matrix= 64×64. The imaging stack of 100 mm did not fully cover the cerebellum and some inferior temporal regions. 120 volumes were acquired for each run of the N-Back task. The first four scans were discarded to allow for magnetic equilibration. In addition, structural scans were acquired using a T1-weighted SPGR sequence for co-registration with fMRI (TE= min full; flip angle, 6°; field of view, 250 mm; bandwidth, 31.25; matrix, 256 x 256; 124 1.3-mm-axial slices). Pre-processing and data analysis were performed using Statistical Parametric Mapping 8 (SPM8 v6313, http://www.fil.ion.ucl.ac.uk/spm) on MATLAB R2012b (The MathWorks, Inc., Natick, Massachusetts, United States) in a Linux environment. After quality check and de-noising obtained using the ArtRepair Software (Mazaika et al. 2009), images were reoriented (without reslice), corrected for slice acquisition time, realigned and unwarped (Andersson et al. 2001; Wilke 2012) using the first image as reference and then co-registered to the individuals' structural T_1 images. Scans were then resampled to 3.75 mm isotropic voxels and

normalized into a standard space (Montreal Neurological Institute, MNI) using a DARTEL template obtained from structural data. Thus, the images were smoothed with a Gaussian kernel of FWHM 10 mm (see Supplemental Material and Methods for a more detailed description of pre-processing). In the first-level analysis, linear contrasts were computed producing a contrast map at each voxel for the 2- > 0- and 3- > 0-Back conditions. Pre-processing and first level GLM modelling were performed separately for the two runs. Both 2-Back and 3-Back blocks were contrasted with 0-Back blocks within runs. Data were scaled at first-level within runs to adjust the signal for potential differences in intensity or in sensitivity to BOLD fluctuations between runs. To partially correct for slice-to-slice movement-induced signal loss, we computed framewise displacement (FD) (Power et al. 2012) and censored the volumes with FD > 0.5 mm in the first level GLM model including a dummy regressor indicating each volume with significant estimated motion. In the bromocriptine study, to investigate the interaction between the PCI, BRO administration and WM load, we computed for each participant and each condition a map of the voxel-wise difference between 3-Back and 2-Back first-level maps using the *imcalc* function provided with SPM8 (http://tools.robjellis.net). Therefore, the difference in BOLD signal between the 3- and 2-Back was our proxy for the neural correlates of inter-individual differences in brain activity during WM. These images entered a second-level analysis using a Flexible Factorial design in SPM8. *Drug* (BRO, PLA) was the repeated measure variable, linear, quadratic, and cubic terms of the PCI entered the model as continuous predictors. Gender was a nuisance factor. In the model design the main effect of subjects, the main effect of *drug*, the interaction between drug and each PCI term (linear, quadratic, and cubic) were computed. Cluster level family-wise error correction for multiple comparisons was performed as implemented in AFNI (-acf function; see (Cox et al. 2017) whole-brain corrected cluster defining threshold k=36, cluster-forming voxel p=.001, FWE-corrected p=.05). Finally, BOLD signal changes

were extracted from significant clusters and pooled using MarsBaR (http://marsbar.sourceforge.net/) for plotting the data. To test the robustness of our analysis reducing the effect of extreme observations we used the bias corrected accelerated bootstrap technique with 10,000 resamples (Efron and Tibshirani 1986). Comparison of fits analysis were performed also on the same BOLD data (Supplementary Materials and Methods, Section 4).

To assess whether drug effects on behaviour and brain activity were related, we computed the difference between BRO and PLA on both behavioural and BOLD signal indices. Then, we assessed a linear model in SPSS in which the dependent variable was the behavioural effect of drug and the independent variable was the BOLD signal; consistent with all other analyses, we included gender in the model as a nuisance factor.

In the replication sample, whose data were pre-processed as in our published report (see (Pergola et al. 2017) for details), we extracted the BOLD signal from the three significant clusters obtained in the bromocriptine study (see results below; these clusters are called regions of interest [ROI] in the following) and computed a repeated measures ANOVA with ROI as within-subjects factor, linear, quadratic, and cubic terms of the PCI as continuous predictors, and gender as a between-subjects factor. Following the results of the bromocriptine study, we estimated the t-parameter of the positive relationship between the quadratic PCI and BOLD variation and computed one-tailed p-values.

Prolactin peripheral levels

Two blood samples (immediately before, and at the end of the fMRI session, respectively) were obtained to measure peripheral prolactin levels. The two prolactin measures served to

test the known effect of BRO on prolactin levels: there is a physiological reduction of prolactin related with circadian rhythms and with the stress induced by the MRI scan (Dunn et al. 1972). Blood prolactin levels are modulated by DA in the tubero-infundibular pathway (Berry and Gudelsky 1991). Thus, we hypothesized an interaction between the PCI and prolactin level changes induced by BRO. For this analysis we considered the entire sample of 71 participants and included gender in the model because of the well-known variation of prolactin peripheral levels in women (McNeilly and Chard 1974). Thus, we computed a repeated measures ANCOVA on prolactin levels, including the within-subject factors *drug* (BRO, PLA) and *measurement* (before or after fMRI), as well as the between-subject factor *gender* and the continuous predictor PCI. We expected a significant *drug* by PCI interaction.

Results

Behavioural data

Differential accuracy was negative and significantly different from zero both on PLA (t_{49}= -4.2, p<.001) and on BRO (t_{49}= -4.1, p<.001), indicating lower performance at 3- compared to 2-back. Repeated measures ANCOVA on behavioural Δ_{WM} index indicated a significant interaction between *drug* and the quadratic term of the PCI ($F_{(1,45)}$= 4.2, p=.046, partial η^2=.085, bootstrapped p=.074). The main effect of *drug* and the *drug*×linear PCI interaction were not significant (p > 0.05). Further behavioural analyses (Supplementary Materials, Section 2) did not identify significant *drug*×quadratic PCI effects irrespective of load, suggesting that the non-linear effect detected was specific to differential accuracy. Figure 1 shows the scatterplot of the interaction. On PLA, very high or very low PCI scores were associated with a larger negative difference between accuracy at 3-Back and 2-Back. This finding implies that people with extreme PCI scores (i.e. with extreme allelic configurations) performed poorer at the highest WM load compared with people with an intermediate PCI score. This pattern of results was reversed on BRO, indicating that individuals with both high and low predicted *DRD2* co-expression showed a larger response to BRO compared with individuals with intermediate PCI scores. Comparison of fits analysis revealed that the quadratic model was preferred to the linear model for this data (Supplementary Material, Section 2). Additionally, the analysis of reaction times revealed a positive relationship between reaction times and linear term of the PCI replicating our previous work (Pergola et al. (2017); see Supplementary Material, Section 2).

The analysis on the replication sample, aimed to replicate the effect identified at placebo in the bromocriptine study, revealed no significant effect of the quadratic term of the PCI

(p>.05).

Figure 1 about here

Imaging data

Table 3 reports the statistics and the localization of clusters of the main imaging results (3-Back > 2-Back activity). We found a significant *drug* × quadratic PCI interaction in the right PFC, particularly in the superior, medial, middle, and inferior frontal gyri (bootstrap p=.006). No clusters were found outside the PFC. There were no other significant findings involving *drug*, PCI and *drug* × linear PCI interaction.

Table 3 about here

Figure 2B shows the scatterplot of the activity estimates extracted from the pooled clusters against the PCI. On PLA, the relationship between the PCI and prefrontal response was U-shaped suggesting that subjects with extreme PCI scores (i.e. with extreme allelic configurations predicting high or low *DRD2* co-expression levels), had a greater positive difference on activation between the two WM loads. In other words, they showed greater prefrontal activity at 3-Back compared to the 2-Back task. BRO administration inverted the U-shaped relationship: participants with extreme PCI scores had a greater negative difference on activation between the two conditions. This finding implies that, after BRO

administration, they had lower prefrontal activity while performing the 3-Back compared to the 2-Back task. Thus, also in this case individuals with high and low predicted *DRD2* co-expression levels showed a similar, large response to BRO. Like in behavioural data, comparison of fits revealed that the quadratic polynomial model was preferred to the linear and to the cubic model also for imaging data (see Supplementary Material and Methods). Further analyses on 2-Back vs 0-Back contrast and 3-Back vs 0-Back contrast separately (Supplementary Materials, Section 3) did not show significant clusters for the *drug*×quadratic PCI interaction, suggesting that the non-linear interaction detected was load-dependent.

The drug effect on prefrontal activity was negatively correlated with the drug effect on behaviour (t_{47}= -2.2, p=.03, partial eta squared=.096), suggesting that the findings in the BOLD response were related with WM performance of participants. The Supplementary Material (Section 3) reports the statistics in the BRO and PLA conditions separately, which revealed that the brain-behaviour correlation was significant at BRO and did not reach statistical significance at PLA.

The analysis on the replication sample, aimed to replicate the effect identified at placebo in the bromocriptine study (Figure 2C), revealed no significant difference between the three ROIs and no significant PCI×ROI interaction. The ROIs derived from the discovery dataset overlapped with the WM network also in this dataset. The positive effect of the quadratic term of the PCI was significant also in the replication sample (t_{47}= 1.9, one-tailed p=.034, partial η^2=.073, bootstrap p=.018).

Figure 2 about here

Prolactin

Repeated measures ANCOVA on prolactin peripheral levels yielded significant main effects of *drug* ($F_{(1,68)} = 28$; $p < .001$; partial $\eta^2=.29$; BRO < PLA), *measurement* ($F_{(1,68)} = 110$; $p < .001$; partial $\eta^2=.62$; first > second), *gender* ($F_{(1,68)} = 85$; $p < .001$; partial $\eta^2=.56$; female > male), and PCI ($F_{(1,68)} = 4.4$; $p = .041$; partial $\eta^2=.06$; positively correlated with prolactin levels). We also found significant *drug*×PCI ($F_{(1,68)} = 4.4$; $p = .04$; partial $\eta^2=.061$), *drug*×*gender* ($F_{(1,68)} = 11$; $p = .002$; partial $\eta^2=.14$), and *measurement*×*gender* ($F_{(1,68)} = 31$; $p < .001$; partial $\eta^2=.32$) interactions. No other main effects or interactions were significant. We resolved the interaction involving the PCI by means of two *post-hoc* partial correlations controlled for *gender* (Bonferroni-corrected $\alpha = .025$) and found that the PCI was associated with prolactin levels specifically on PLA ($r = .28$, $p = .021$), but not on BRO ($r = .094$, $p = .44$). The positive correlation indicates that higher predicted *DRD2* expression levels were associated with higher prolactin levels (Figure S2).

Discussion

The present study aimed to investigate in healthy humans whether allelic patterns predicting the co-expression of a *DRD2* gene set are associated with PFC function during WM to D_2R stimulation. We found that the PCI predicting *DRD2* co-expression interacted with BRO administration on i) WM differential accuracy ii) DLPFC activity during WM, and iii) prolactin peripheral levels. Furthermore, consistently with our hypothesis, the effect of BRO on Δ_{WM} depended non-linearly on the PCI. These findings suggest that drug response to BRO co-varies with variation in multiple genes co-expressed with *DRD2* (Pergola et al. 2017). Notably, different alleles in the same SNPs reflecting high or low *DRD2* transcription levels were associated with *similar* behavioural and brain outcome, i.e., increased Δ_{WM} and decreased DLPFC activity.

Prefrontal function during working memory

Both imaging and behavioural findings are consistent with previous studies reporting a modulatory effect of BRO on prefrontal function during WM (Kimberg et al. 2001; Gelao et al. 2014). Moreover, BRO administration interacts non-linearly with the PCI in both behavioural and fMRI analyses. Importantly, the behavioural pattern observed was opposite to the BOLD pattern, i.e., BOLD increase in the DLPFC was paralleled by poorer performance. This finding was further supported by the inverse correlation between behaviour and brain response. The inverse correlation can be interpreted in the context of WM efficiency (i.e. the amount of neural resources recruited for a certain level of performance; (Manoach 2003; Bertolino and Blasi 2009). According to the efficiency model, an inefficient response is characterized by low performance paralleled by increased

investment of neural resources, consisting in greater brain activity or recruitment of additional brain areas (Callicott et al. 2003; Van Snellenberg et al. 2015). The significance of the correlation appeared driven by the BRO condition. This result may be interpreted as reflecting a drug effect, but it is difficult to reach a conclusion at the current stage based only on the negative finding in the PLA condition.

In this study, individuals with extreme allelic patterns, i.e., having either low or high predicted co-expression of the gene set including *DRD2* in the DLPFC, exhibited similar response to D_2R stimulation compared with intermediate allelic configurations. In particular on PLA, individuals with extreme genotype configurations had a decrease in differential accuracy and an increase in BOLD response during 3-Back with respect to 2-Back, reflecting a phenotype of inefficiency. It is important to note that these findings have been obtained in healthy subjects likely reflecting the physiological portion of the inverted-U curve. This finding also follows the inverted U-shaped relationship between WM processing and DA-signalling (Cools and D'Esposito 2011). (Seamans and Yang 2004) reviewed evidence on D_1 receptors strongly supporting this model and also noted that PFC D_2 receptors effectively exert opposite effects with respect to D_1-mediated activity. Based on such a model, while individuals with allelic patterns predicting intermediate co-expression levels of the *DRD2* gene set display optimal WM processing, individuals with extreme alleles may be at a disadvantage because of insufficient or excessive D_2-related activity. For example, individuals with high *DRD2* gene set co-expression in the DLPFC may be less efficient in terms of WM processing reflecting a proneness to a D_2-status characterized by low signal to noise ratio (Kahnt et al., 2015; Seamans and Yang, 2004). Given the strong relationship between executive function and WM capacity (McCabe et al. 2010; Johnson et al. 2013), this

phenotype of inefficiency may be associated with WM capacity limits. These findings highlight that the model put forward by (Seamans and Yang 2004) of a D_2-dominated status is associated with the co-expression of a gene set, and not just with the *DRD2* gene. Interestingly, this pattern was reversed following BRO administration. This finding suggests that healthy people with allelic patterns reflecting high or low co-expression of the *DRD2* gene set associated with inefficient WM processing were the same who benefitted most from the effect of D_2R stimulation with BRO.

It is difficult to put forward a mechanistic explanation of this "bidirectional" effect of BRO. One might expect that individuals with low PCI (lower predicted *DRD2* co-expression) could benefit more from a D_2R agonist than individuals with high PCI (higher predicted *DRD2* co-expression). Instead, our findings show that individuals with low WM capacity, whether associated with high or low PCI, showed greater improvement under BRO than those with intermediate PCI values. These findings are consistent with evidence from animal (Marighetto et al. 2008; Tarantino et al. 2011) and human (Kimberg et al. 1997) studies, and with previous reports showing that individuals carrying alleles associated with phenotypes of inefficiency are likely to show a higher magnitude of drug effect (Bertolino et al. 2004; Blasi et al. 2013; 2015).

To explain why a D_2R agonist in the current data appeared to act as a buffering agent (i.e., acting to the benefit of most extreme genotypes in both directions of the curve), it should be considered that the PCI has been developed as an index of mRNA co-expression in the DLPFC, thus it approximates post-synaptic *DRD2* mRNA (primarily the long isoform; Pergola et al. 2017), together with the expression of its partner genes. However, BRO also acts on presynaptic receptors, which are not monitored by the PCI. Moreover, the PCI does not index either DA levels or D_1, both involved in WM modulation. In our previous work, we

reported that the *DRD2* co-expression gene set was functionally enriched for "negative regulation of dopamine secretion" (GO:0033602). On this account, it is possible that the transcriptomic context of *DRD2* exerts an effect on DA levels, and further biological experiments are warranted to test this hypothesis at cell level. Finally, the co-expression module is not a proxy for only post-synaptic D_2 receptors, but accounts for the co-expression of many other genes which may or may not be sensitive to DA neurotransmission – this remains to be tested.

In our previous work (Gelao et al. 2014) we showed that a *single* functional SNP within the *DRD2* gene predicted brain and behavioural effects of bromocriptine during WM processing. In the present study, we showed that that multiple alleles that co-vary with *DRD2* expression are associated with brain and behavioural response to D_2R stimulation. As in our previous study (Pergola et al. 2017), the PCI effect persisted when we covaried by rs1076560 (data available upon request). Notably, we did not observe a main effect of drug on differential accuracy, thus BRO administration in this study appeared to be either advantageous or detrimental depending on a complex, polygenic background of individuals. This finding is particularly relevant to pharmacogenomics because it supports the idea that allelic patterns, rather than specific alleles, may be associated with phenotypes of clinical interest, such as drug effect on brain processing.

DRD2 PCI is associated with prolactin peripheral levels

The findings on prolactin peripheral levels are consistent with the well-known effect of D_2R agonists on prolactin peripheral levels (Berry and Gudelsky 1991). Since the PCI is based on DLPFC gene expression data, this finding may suggest that the genetic variants detected by (Pergola et al. 2017) affect DA signalling on multiple brain sites, and not only in the DLPFC.

However, further studies are needed to test this hypothesis more specifically. These findings are also in line with the evidence that genetic markers can be used to stratify prolactin response in patients (Sukasem et al. 2016) and support the idea that the SNPs included in the PCI predict *DRD2* transcription. This is of particular interest for the treatment of psychosis because hyperprolactinemia and related sexual dysfunctions are common side effects of D_2R blockade exerted by antipsychotics (Peveler et al. 2008). One limitation of this finding is that we did not control for menstrual phase. Although we attempted to control for this confound by including the factor gender in all analyses, this limitation may undermine the signal to noise ratio in females.

Taken together, the present findings suggest that a gene set co-expressed with *DRD2* and indexed by the PCI is associated with multiple D_2R-dependent phenotypes, also including systemic response to pharmacological challenge.

Limitations

BRO targets multiple receptors, and not only the D_2Rs (Sautel et al. 1995). Furthermore, BRO exerts its effect on both short and long isoforms of D_2R (Gardner and Strange 1998; Gelao et al. 2014), while the PCI is primarily associated with D_2 long-specific mRNA. Thus, it is not possible to elaborate a synaptic model of how BRO interacts with the PCI on the phenotypes we considered. While the bromocriptine study is well-powered compared to other similar studies (Kimberg et al. 2001; Bloemendaal et al. 2015), a sample size of 50 individuals is small in the context of a genetic study (Casey et al. 2010). For this reason, we avoided excluding participants with extreme values, because they represent extreme genotypic configurations. Furthermore, the behavioural effects here reported have a moderate size which is comparable with similar findings with polygenic approaches (Cohen 1988;

Pergola et al. 2016), but greater samples are likely required to resolve the ambiguity of the behavioural results, which were not significantly replicated in the second sample. Importantly, we used differential accuracy as an index of WM capacity, reflecting more consistent accuracy in the face of load increase. Since the quadratic PCI effect was not found on accuracy scores irrespective of load, the present study does not provide direct evidence of an inverted-U relationship on WM accuracy indexed as percentage of correct responses.

Sample size may also affect the fMRI results. In particular, we found that the repeated measures models we employed were more sensitive to artefacts compared to simpler models (e.g., the single session design of the replication dataset). Therefore, we employed correction tools for the main analysis (Mazaika et al., 2009) and a pre-processing pipeline designed to minimize signal loss. To discount the risk of type I error possibly associated with artefact correction, we sought replication of the results in our previously published dataset (Pergola et al. 2017). We found that the effect identified at placebo was significant also in this additional dataset.

Conclusion

The present findings suggest that the role of DA signalling mediated by D_2R in WM is associated with genetic variants in multiple genes co-regulated with *DRD2*. Together with the results of our previous study, the present evidence reveals both linear and non-linear relationships between genetically predicted *DRD2* co-expression and brain and behavioural correlates of WM. Non-linear effects only emerged in the present study when load differences were taken into account. Inter-individual variation in WM brain/behavioural response takes the form of an inverted U-shaped relationship with predicted *DRD2* co-expression levels that is reversed when individuals are challenged with BRO. Importantly, this implies that individuals with opposite allelic patterns can show similar drug response, depending on the downstream physiological correlates of genetic variation. In conclusion, the present work provides novel information on the genetic architecture of WM capacity and related PFC activity and encourages both pharmacogenomics and imaging genetics to study allelic patterns associated with molecular phenomena such as gene co-expression to predict drug response.

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Table 1. Sociodemographic characteristic of the three samples (mean ± SD). IQ: intelligent quotient, WAIS-R: Wechsler Adult Intelligent Scale-Revised.

	N	Gender (males)	Age (years)	IQ (WAIS-R)	Socioeconomic status (Hollingshead Scale)	Handedness (Edinburgh Inventory)
Prolactin study	71	34	26±5.1	111±12	46±16	0.73±0.42
fMRI and Behavioural studies (Discovery)	50	27	27±4.1	113±12	47±16	0.77±0.35
Replication dataset	50	28	27±7.0	111±12	46±13	0.89±0.12

Table 2. Single Nucleotide Polymorfisms (SNPs) used to compute the PCI. MAF: minor allele frequency in the Braincloud sample. LD: Linkage disequilibrium. SNPs in Linkage Disequilibrium were identified in the 1000 Genome Pilot 1 dataset with SNP Annotation and Proxy Search (SNAP, Broad Institute, http://archive.broadinstitute.org/mpg/snap/, and the following criteria: R^2 threshold= 0.8; search window= 500 kbp, Caucasian sample).

Rank	Marker	Locus	Gene	Gene name	Position	SNPs in LD	MAF
1	rs2486064	1q32.1	CHIT1	Chitinase 1	chr1:203199636	-	0.22
2	rs6902039	6p22.3	GPLD1	Glycosylphosphatidylinositol Specific Phospholipase D1	chr6:24583953	-	0.23
3	rs851436	2p24.1	OSR1	Odd-Skipped related 1	chr2:19283340	rs851435 rs851434 rs851433 rs2881717 rs851438 rs851439 rs851351 rs1658258 rs1727212 rs851353 rs851354 rs851358 rs851361 rs851365 rs851367 rs851370 rs851371 rs703324 rs851369 rs851437 rs1100943	0.48
4	rs9297283	8q22.2	POP1	Processing Of Precursor 1, Ribonuclease P/MRP Subunit	chr8:98154668	-	0.20

5	rs12940715	17q25.1	SDK2	sidekick cell adhesion molecule 2	chr17:73481592	-	0.20
6	rs1805453	17p13.2	DHX33	DEAH (Asp-Glu-Ala-His) Box Polypeptide 33	chr17:5482078	rs28372907 rs3888575 rs9891023 rs9910302 rs9912170 rs8081261 rs8082510 rs8082665 rs9893583 rs9900370 rs9906174 rs9901132 rs9907870 rs9907789 rs58054077 rs9915716 rs9916398 rs9895207 rs1805430	0.34
7	rs11213916	11q22.3	BTG4	B-Cell Translocation Gene 4	chr11:111468254	rs11213918 rs9783376 rs10891273 rs4938639 rs35181851	0.30
8	rs1037791	7p21.1	AGR2	Anterior Gradient 2	chr7:16785037	rs6970366 rs1997116 rs2272246 rs13242497 rs7796640	0.31

Table 3. Statistics of the FWE-corrected clusters for the $PCI_{quadratic}$×*drug* interaction at a cluster-defining threshold of k = 36 (cluster-forming voxel p= 0.001; FWE-corrected p= 0.05).

Cluster	Cluster extent	Region	Brodmann Area	MNI coordinates (x, y, z)	F	Z
1	93	Right Medial Frontal Gyrus	Right BA8	4, 34, 42	44.51	5.43
		Right Superior Frontal Gyrus	Right BA6	4, 19, 58	20.74	3.95
2	36	Right Middle Frontal Gyrus	Right BA44	38, 16, 24	18.68	3.77
3	56	Right Inferior Frontal Gyrus	Right BA47	42, 30, -6	17.97	3.70

Captions to figures

Figure 1. Scatterplot of the *drug* by the *quadratic term of the Polygenic Co-Expression Index (PCI)* interaction on behavioral data. On the Y axis, Δ_{WM} index refers to the unstandardized residuals of the model (see text). The X axis illustrates the PCI, which is directly proportional to predicted *DRD2* gene set co-expression. On Placebo, individuals with extreme genotype configurations, i.e., with either low or high predicted *DRD2* expression in the dorsolateral prefrontal cortex (DLPFC), exhibited a greater decrease in accuracy during 3-back with respect to 2-back compared to subjects with intermediate PCI. This pattern was reversed following Bromocriptine administration.

Figure 2. (A) Sections of the brain showing the significant clusters in which the *drug* by *quadratic term of the Polygenic Co-Expression Index (PCI)* interaction was found. **(B)** Scatterplots of the BOLD measure (unstandardized residuals) extracted from the pooled prefrontal clusters against the PCI, showing the quadratic relationship and the inversion of the pattern after Bromocriptine administration. **(C)** Scatterplots of the BOLD measure (unstandardized residuals) extracted from the replication data against the PCI, showing the replication of the quadratic relationship independent on drug or placebo administration.